Refining the sensory and motor ratunculus of the rodent upper extremity: evaluation of the C7 nerve root using fMRI and direct nerve stimulation

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Abstract

Background Since the 1980s, the C7 nerve root has gained clinical relevance as a donor nerve in severe brachial plexus root avulsion injuries. Despite success with the cross-chest C7 nerve transfer, inducing injury on an otherwise normal side hinders global acceptance. By sacrificing the C7 nerve root, a predictable pattern of transient sequelae is seen, including extensor weakness and index and middle finger anesthesia. The purpose of this study is to observe cortical activity during direct stimulation of the C7 nerve root using blood oxygen level dependent functional magnetic resonance imaging (fMRI) in a rat model.

Methods A total of 12 male Sprague-Dawley rats, weighing 200–250 g, were used in this study. Following an acclimation period of 1 week, 12 rats underwent exposure and dissection of the brachial plexus. Seven rats underwent placement of an implantable electrode (AISI 304, Plastics1, Roanoke, VA, USA) on the C7 nerve root, while five rats underwent electrode placement on the radial nerve. All animals then underwent fMRI during direct nerve stimulation. Ten consecutive coronal images were obtained during nerve stimulation, using a 9.4-T small-animal MRI scanner. **Results** Cortical activation is seen within a very specific area of the primary sensory region of the forelimb during

C7 nerve root stimulation. The cortical activation seen during radial nerve stimulation includes that seen during C7 stimulation but extends several slices caudally.

Conclusions The sensory representation of the C7 nerve root is seen in only a small area in the S1FL region compared to that seen in the terminal branches of the brachial plexus. However, this area shows a significant overlap with the S1FL area of activation seen during radial nerve stimulation. This makes sense as the C7 nerve root contributes some, but not all, sensory axons to the radial nerve. Mapping of the C7 cortical representation in the rat brain not only adds to the ongoing development of the motor and sensory ratunculus but also provides an important foundation to study subsequent C7 donor nerve models.

Keywords C7 nerve root · Rodent MRI · Cortical plasticity

Introduction

The brachial plexus is a complex group of nerves originating from the roots of C5–T1, providing the upper extremity with motor and sensory function. While injuries have been sustained throughout history, only in the last century has technology been such that repair is possible. In the case of severe root avulsion injuries, multiple nerves have been identified as possible donors, including the C7 nerve root [7]. However, despite over 20 years of clinical experience with the cross-chest C7 nerve root transfer, outcomes remain inadequate with only 28–54% of patients achieving a motor score of M3 or better [6, 8, 9]. What is not known is whether the inadequacy of repair occurs at the level of the peripheral nerve, the spinal cord, or the cerebral cortex. Since 1992, blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) has provid-

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ed a reliable method for indirectly studying task-induced cerebral neuronal activity [21]. More recently, BOLD fMRI has been applied to the study of cortical plasticity following nerve injury and repair [22]. The first step in developing a rodent fMRI survival model of the cross-chest C7 nerve root transfer is identifying the cortical representation of the C7 nerve root.

It is well understood that the body surface is portrayed in cortical and subcortical fields in a highly organized topographical map [10–14, 17–20]. Although initially studied with invasive electrophysiological methods, more recently BOLD fMRI has been used to help identify these cortical topographical maps during peripheral stimulation [1, 15]. Simply stated, BOLD fMRI is an imaging modality which indirectly measures neuronal activity through a signal generated by oxygenated hemoglobin. When a peripheral stimulus is applied, there is increased neuronal activity in the corresponding cortical territory. In turn, this results in increased metabolic demand, leading to vasodilatation through autoregulatory mechanisms. The increased blood flow exceeds that of metabolic demand, yielding oxygenated blood within the venous microcirculation. Oxygenated blood is diamagnetic, as opposed to de-oxygenated blood, which is paramagnetic [23]. The increased ratio of oxygenated to de-oxygenated blood leads to an increased BOLD signal (see Fig. 1).

Because of the homology in the rat and human peripheral nervous systems, the rat has provided an excellent model for the study of human upper extremity function (see Fig. 2) [2, 3]. Furthermore, as the homunculus has been described in the human, so too has the ratunculus been described in the rat. In 2008, Cho et al. identified the cortical activity associated with the terminal branches of the brachial plexus in a rat population using

fMRI and direct nerve stimulation [4, 5]. During these studies, large areas of cortical activation were identified while an electrical current was applied to each terminal branch of the plexus. As each terminal branch receives contributions from multiple nerve roots, theoretically a smaller, more specific area of cortical activation should be seen when stimulating more proximally within the brachial plexus. In this study, we further define the ratunculus by identifying the cortical representation of the C7 nerve root using fMRI and direct nerve root stimulation.

Materials and Methods

Animal Requirements All protocols and procedures in this study were carried out in compliance with federal regulations and the guidelines of the Medical College of Wisconsin's Institutional Animal Care and Use Committee. A total of 12 male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used in this study.

General Sequence Following an acclimation period of 1 week, 12 male Sprague-Dawley rats, weighing 250–300 g, were separated into two groups (7=C7 nerve stimulation, 5=radial nerve stimulation). Group I rats underwent fMRI during direct electrical stimulation of the right C7 nerve root. Group II rats underwent fMRI during direct electrical stimulation of the right radial nerve.

Nerve Operation All 12 animals were subjected to nerve surgery following acclimation. To induce general anesthesia, the animal was first placed in a transparent chamber, where Isoflurane (1.4%, Halocarbon Laboratories, River Edge, NJ, USA) was administered through a vaporizer. The

Fig. 1 **a** Schematic of BOLD fMRI. **b** Peripheral nerve stimulation leading to cortical activation shown

Fig. 2 Cartoon and photograph demonstrating homology between **a** human and **b** rat plexus

animal was then transferred to a heated operating table where isoflurane was continuously administered through a nosecone. After being placed in the supine position, the right neck and axilla were clipped with an electric razor. The area was then prepped with povidone–iodine and draped in sterile fashion. To begin the procedure, a 2.0-cm incision was placed longitudinally in the right neck, extending obliquely into the right axilla. The subcutaneous tissues were then divided to the level of the pectoralis major. The brachial plexus was then identified at the level of the terminal branches, immediately lateral to the border of the pectoralis major muscle. For animals undergoing C7 nerve electrode placement, the pectoralis major muscle was bluntly divided parallel to its fibers. The nerve roots were then identified deep to the muscle. Following identification, the C7 nerve root was isolated, and a 125- μ m-diameter stainless steel bipolar electrode (Plastics 1, Roanoke, VA, USA) was placed circumferentially around the nerve root (see Fig. 3). The bipolar electrodes used are coated with polymide insulation. The polymide coating was removed only on the surface of the electrode in direct contact with the nerve being tested, thereby shielding adjacent nerves. For animals undergoing radial nerve electrode placement, the dissection was

performed at the level of the terminal branches of the plexus. The radial nerve was identified deep to the median and ulnar nerves. After isolation of the nerve, an electrode was placed circumferentially around the radial nerve (see Fig. 3). All nerve dissections and electrode placements were performed with the use of the operating microscope. Following electrode placement, the overlying skin was closed using interrupted 3–0 nylon sutures. The pedestal of the implantable electrode was left exposed through the skin. A cable was attached to the pedestal to allow for direct nerve stimulation. At this point, venous access was obtained using direct cut-down to the right femoral vein. Tracheostomy was then performed to allow for mechanical ventilation during scanning. Immediately following the operation, the animal was transferred to the Bruker 9.4-T small-animal MRI scanner, where fMRI was performed during direct nerve stimulation.

Anesthesia Following the surgical procedure, once the animal was transferred to the MRI scanner, isoflurane was discontinued and dexmedetomidine (Orion Corp., Espoo, Finland) was administered intravenously at a rate of 100 mcg/kg/h, along with pancuronium bromide (Hospira, Inc., Lake Forest,

Fig. 3 **a** Close-up photograph of implantable electrode placed on the C7 nerve root. **b** Close-up photograph of implantable electrode placed on the radial nerve

IL, USA) at rate of 2 mg/kg/h [24]. Once scanning was complete, the animal was euthanized using Nembutal (Schering-Plough Animal Health Corp., Union, NJ, USA) 120 mg/kg given intravenously.

Functional MRI Parameters The BOLD response to nerve stimulation in the primary sensory and motor regions was studied. A rapid acquisition with relaxation enhancement (RARE) anatomic image was obtained prior to each fMRI

Fig. 4 **a** Once the electrode is placed, the animal is transferred to the 9.4-T small-bore animal MRI scanner. **b** During scanning, a block design nerve stimulation protocol is performed as shown. **c** Ten contiguous coronal images of the cortex are obtained during nerve stimulation. Slice 3 is located immediately over the anterior commissure, located 0.36 mm posterior to bregma

experiment. RARE parameters were repetition time (TR)=2.5 s, echo time (TE)=50.8 ms, field of view (FOV)=35 mm, and a 256×256 matrix. Gradient recalled echo-planar scans were acquired using a Bruker AVANCE 9.4-T MRI scanner (AVANCE, Bruker, Billerica, MA, USA) with a 30-cm bore. Images were acquired using a Bruker receiving surface coil (T9208) and a linear transmit coil (T10325). Ten 1-mm-thick contiguous slices were acquired, with slice 3 located directly over the anterior commissure (−0.64 mm from bregma). This slice profile allowed adequate coverage of the sensorimotor system. A T^2 -weighted echo-planar imaging (EPI) sequence was used for the fMRI experiments. EPI parameters were TR=2 s, TE=18.76 ms, FOV=35 mm, and a 96×96 matrix (zero filled to a 128×128 matrix), with the same slice geometry as the RARE images. One hundred ten images were obtained during each fMRI experiment for a total acquisition time of 3 min 40 s.

Functional MRI Electrical Stimulation Method A square-wave electrical generator (S88, Grass Telefactor, Warrick, RI, USA) equipped with a constant current unit (Grass Telefactor, Warrick, RI, USA) was used for all nerve stimulations during fMRI scanning. A standard

block design stimulation protocol of 40-s rest followed by three periods of 20 s on and 40 s off was used (total of 3 min 40 s; see Fig. 4). Electrical stimulation was computer-controlled and triggered by a transistor–transistor logic pulse from the scanner. Direct nerve stimulation was carried out with a fixed current of 0.5 mA, frequency of 10 Hz, and duration of 1 ms.

Functional MRI Data Analysis All EPI acquisitions were then registered to an “ideal” RARE image using the Oxford Center for Functional Magnetic Resonance of the Brain’s Linear Image Registration Tool program. The EPI datasets were used to create BOLD activation maps. The activation maps are created by performing an F test on the time series with the block design as the only regressor (AFNI, 3dDeconvolve). Activation was determined using a p value threshold of 0.005 (using AFNI). Statistical significance for the number of activated voxels was determined using an unpaired t test with a p value of <0.05.

Physiologic Monitoring During MRI Image Acquisition During all MRI scanning sessions, the rat was allowed to breathe spontaneously through a nosecone with 30% FIO₂. While scanning, chest respiration (Model 1025, SA

Fig. 5 fMRI results during C7 nerve root stimulation. **a** Ten coronal slice profiles obtained during fMRI. **b** Slice, located approximately 0.36 mm anterior to bregma, with activated area of S1F1 region

highlighted. **c** Ten coronal slices obtained during direct stimulation of the C7 nerve root. Note activation within the S1F1 region in slice 2

Instruments, Stony Brook, NY, USA), end tidal gases (POET IQ2, Criticare Systems, Waukesha, WI, USA), and pulse oximetry (Model 8600 V, Nonin Medical, Plymouth, MN, USA) were continuously monitored.

Results

A total of 12 rats underwent implantable electrode placement (radial nerve=5, C7 nerve root=7), followed by fMRI during direct nerve stimulation. During all scanning, mean temperature was 34.86°C (range 32.1–37.3°C), mean pulse was 267.45 bpm (range 206–365 bpm), mean pulse oxymetry was 94.63% (range 88–99%), and respirations were set at 65. There were no fatalities during scanning.

Figure 5 demonstrates fMRI results during stimulation of the right C7 nerve root. In Fig. 5a, the coronal slice configuration is demonstrated over a single sagittal cut, orienting the ten coronal slices which lie over the sensorimotor cortex. Figure 5c shows the ten coronal slices obtained during fMRI. Slice 0 is most cranial and slice 9 is most caudal. The view of the coronal slices is caudocranial, with the left cerebral hemisphere lying on the left side and

the right cerebral hemisphere lying on the right side. Figure 5c demonstrates the average voxel activation during stimulation of the right C7 nerve root for all seven animals. Only statistically significant voxel activation is shown, as described in the “Materials and Methods” section. During stimulation of the C7 nerve root, a focal area of activation is identified in slice 2, located approximately 0.64 mm anterior to bregma. Average voxel activation during right C7 nerve root stimulation measured 51.71 voxels in the contralateral cerebral cortex (range 40–65, SD=9.39). When this activation territory is applied to the appropriate plate of the Paxinos and Watson rat brain atlas (Fig. 5b), the area of activation is identified within the primary sensory region of the forelimb (S1F1).

Figure 6 demonstrates fMRI results during stimulation of the right radial nerve. Figure 6a again shows the coronal slice configuration. Figure 6b shows the ten coronal slices obtained during fMRI. The orientation of the coronal slices is the same as that described previously, with the left cerebral hemisphere lying on the left side and the right cerebral hemisphere lying on the right side. Figure 6b demonstrates the average voxel activation during stimulation of the right radial nerve root for all five animals. Only statistically significant voxel activation is shown, as described in the

Fig. 6 fMRI results during radial nerve stimulation. **a** Ten coronal slice profiles obtained during fMRI. **b** Ten coronal slice obtained during direct stimulation of the radial nerve. Note the activation within

the S1F1 region extending from slice 2 caudally to slice 6. Applied to each slice is the corresponding plate from the Paxinos and Watson rat brain atlas

“Materials and Methods” section. During stimulation of the radial nerve, there is a much broader area of activation. The activation begins in slice 2 (bregma+0.36 mm) but extends continuously for 4 mm in a caudal direction, with activation extending into slice 6, located 3.64 mm posterior to bregma. Average voxel activation during right radial nerve stimulation measured 295.6 voxels in the contralateral cerebral cortex (range 274–322, SD=18.23). The difference in voxel counts is statistically significant ($p<0.001$) when comparing radial nerve activation to C7 nerve activation. When the appropriate plates of the Paxinos and Watson brain atlas are applied, again the activation is located within the primary sensory region of the forelimb. Interestingly, when comparing radial nerve activation to C7 nerve root activation, there is a nearly identical pattern of activation located within slice 2.

Discussion

Within the normal layout of the brachial plexus, the C7 nerve root lies in the middle, with two nerve roots cephalad and two caudal. As a result of redundancy and overlap within the plexus, the contralateral C7 nerve root has been identified as an axon donor in the setting of severe root avulsion injury. The cross-chest C7 nerve root transfer directs healthy axons from the uninjured upper extremity to

the injured upper extremity, with the goal of improving function [7–9]. Despite two decades of experience with this procedure, outcomes continue to produce unpredictable results [6]. What remains to be understood is why such disparity exists in this patient population. Whether it is a problem of cortical reorganization or peripheral nerve regeneration is unknown. BOLD fMRI has recently been employed to study cortical plasticity following nerve injury and repair. The first step in developing an fMRI model of the cross-chest C7 nerve root transfer is identifying the cortical territory supplied by the C7 nerve root. In this study, building on previous works in our laboratory, we further define the ratunculus by identifying the cortical territory supplied by the C7 nerve root.

In histological studies, the posterior division of the C7 nerve root has been shown to supply approximately 50% of its axons to the radial nerve, making it the single greatest contributor to that nerve [6]. However, the radial nerve also receives contributions from the C5 nerve root, the C6 nerve root, the C8 nerve root, and occasionally the T1 nerve root. When stimulating at the level of the radial nerve, we see a large area of cortical activation, extending from slice 2 caudally to slice 6. This spans a large area of the S1F1 region. Interestingly, as we move proximally within the brachial plexus and stimulate the C7 nerve root, we see a much more focal territory of activation, located only in slice 2. As a contributor to the radial nerve, we would expect an

Fig. 7 **a** BOLD fMRI activation seen during digital stimulation. **b** BOLD activation during right C7 nerve root stimulation. **c** Corresponding atlas plate showing S1F1 region *highlighted*

overlapping pattern when comparing the cortical territories of the C7 nerve root and the radial nerve. When looking at the activation pattern in slice 2, we see nearly an identical territory of activation for both the C7 nerve root and the radial nerve.

In human patients, the donor limb sequelae following C7 nerve root transection have been well described [6–9]. Typically, following C7 nerve root transection, patients complain of transient index and middle finger numbness and paresthesias, along with extensor weakness. In our lab, using high-resolution fMRI, we have recently identified the cortical territories associated with single digital stimulation in a rat [16]. Interestingly, when electrically stimulating the second and third digits (analogous to the index and middle fingers, respectively), we see a very similar distribution of cortical activation as compared to that seen during C7 nerve root stimulation (see Fig. 7). This would suggest strong homology of the C7 nerve root in the human and rat and implicate the rat as an appropriate model for the study of cross-chest C7 nerve root transfer. By identifying the cortical activation associated with C7 nerve root stimulation, we not only add to the ongoing development of the ratunculus but we also create a foundation for the study of subsequent C7 donor nerve models.

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Conflicts of interest The authors have no conflicts of interest to declare.

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